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DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L8	l7 and two step$1	0	L8
L7	L6 and (fluorescen$2 or ethidium)	15	L7
L6	immuno same polymerase same reverse transcript$3	23	L6
L5	immuno same polymerase same revers transcript$3	0	L5
L4	immono same polymerase same reverse transcript$3	0	L4
L3	l2 and ethidium	0	L3
L2	L1 and fluorescen$2	2	L2
DB=DWPI,USPT,EPAB,JPAB; PLUR=YES; OP=ADJ			
L1	5922553.pn.	2	L1

END OF SEARCH HISTORY

Generate Collection

L7: Entry 11 of 15 File: USPT May 25, 1999

DOCUMENT-IDENTIFIER: US 5907085 A
TITLE: Grapevine leafroll virus proteins and their uses

Drawing Description Text (5):
FIGS. 4A and 4B, panel B, is a Northern blot hybridization. Probe made from a clone insert gave positive reaction to itself (lane 3) as well as dsRNA from leafroll infected tissues (lane 1), but not to nucleic acids extracted from healthy grapevines (lane 2). Lane M contains a molecular weight marker (the Hind III digested fragments of lambda DNA). Panel A of FIG. 4 depicts an ethidium bromide stained agarose gel before transfer to a membrane.

Drawing Description Text (6):
FIG. 5 presents an analysis of GLRaV-3 dsRNA by electrophoresis on an ethidium bromide stained agarose gel. A dsRNA of ca. 16 kb was readily isolated from diseased grapevine (lane 6), but not from the healthy control (lane 5). Other samples that were used for control were tobacco mosaic virus dsRNA (lane 1); cucumber mosaic virus dsRNA (lane 2); pBluescript vector (lane 3) and an insert of clone pC4. .lambda. Hind III digested fragment of lambda DNA was used as the molecular weight marker (lane M).

Detailed Description Text (73):
Antibodies raised against the proteins or polypeptides of the present invention or binding portions of these antibodies can be utilized in a method for detection of grapevine leafroll virus in a sample of tissue, such as tissue from a grape scion or rootstock. Antibodies or binding portions thereof suitable for use in the detection method include those raised against a helicase, an RNA-dependent RNA polymerase, an hsp70-related, an hsp90-related, or a coat protein or polypeptide in accordance with the present invention Any reaction of the sample with the antibody is detected using an assay system which indicates the presence of grapevine leafroll virus in the sample. A variety of assay systems can be employed, such as enzyme-linked immunosorbent assays, radioimmunoassays, gel diffusion precipitin reaction assays, immunodiffusion assays, agglutination assays, fluorescent immunoassays, protein A immunoassays, or immunoelectrophoresis assays.

Detailed Description Text (137):
PCR technology has been applied to detect viruses, viroids and phytoplasmas in the field of plant pathology (Levy et al., "Simple and Rapid Preparation of Infected Plant Tissue Extracts for PCR Amplification of Virus, Viroid and MLO Nucleic Acids," Journal of Virological Methods, 49:295-304 (1994), which is hereby incorporated by reference). However because of the presence of enzyme inhibitors (reverse transcriptase and/or Taq DNA polymerase) in many plant tissues, a lengthy and complicated procedure is usually required to prepare a sample for PCR. In studies of PCR detection of grapevine fanleaf virus, Rowhani et al., "Development of a Polymerase Chain Reaction Technique for the Detection of Grapevine Fanleaf Virus in Grapevine Tissue," Phytopathology, 83:749-753 (1993), which is hereby incorporated by reference, have already observed an enzyme inhibitory phenomenon. Substances such as phenolic compounds and polysaccharides in grapevine tissues were suggested to be involved in enzyme inhibition. Present work further confirmed this observation. One of the objectives in the present study was to develop a sound practical procedure of sample preparation to eliminate this inhibitory problem for PCR detection of GLRaV-3 in grapevine tissues. Although the expected PCR product was consistently observed from samples of dsRNA, purified virus and partial purified virus, proteinase K-treated crude extract and immuno-capture methods were the simplest and were still effective. Samples prepared with proteinase K-treated crude extract have an advantage over others in that hazardous organic solvents, such as phenol and chloroform, are avoided. However, care must be taken in the sample concentration because the reaction can be inhibited by adding too much grapevine tissue (see lane 3 in FIG. 14). Minafra et al., "Sensitive Detection of Grapevine

Virus A, B, or Leafroll-Associated III from Viruliferous Mealybugs and Infected Tissue by cDNA Amplification," Journal of Virological Methods, 47:175-188 (1994) ("Minafra (1994)"), which is hereby incorporated by reference, reported the successful PCR detection of grapevine virus A, grapevine virus B, and GLRaV-3 with crude saps prepared from infected grapevine tissues, this method of sample preparation was, however, not effective in the present study. The similar primers used by Minafra (1994), which is hereby incorporated by reference, were, however, able to amplify the expected size of PCR products from dsRNA of the NY1 isolate of GLRaV-3.